Fungal Cleavage of the Sterol Side Chain

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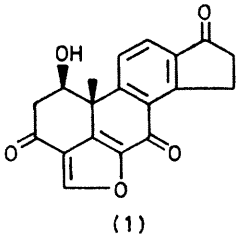
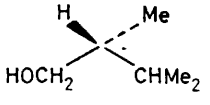
Summary A group of alcohols with the structure of the sterol side chain have been isolated from *Nodulisporium hinnuleum* and shown to have a specific activity when biosynthesized from [2-^{14}C]mevalonic acid, consistent with a common origin with their co-metabolite, the 17-keto-steroid demethoxyviridin.

THE bacterial cleavage of the side chain of sterols is different from that in mammals. In the latter, cleavage of the C(20)–C(22) bond with the formation of isohexanal is followed by cleavage of the C(17)–C(20) bond to give the 17-keto-steroids,[1] whilst micro-organisms of the genera *Nocardia*, *Arthrobacter*, *Mycobacterium*, and *Corynebacterium* commonly shorten the side chain by a pathway comparable with that of the oxidation of fatty acids to give propionic and acetic acids.[2] Demethoxyviridin (**1**), obtained from *Nodulisporium hinnuleum*, is a fungal 17-keto-steroid related to viridiol which is biosynthesized *via* squalene.[3] It was therefore of interest to see if the removal of the side chain followed the mammalian or bacterial pattern.



(1)



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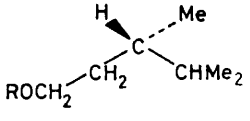
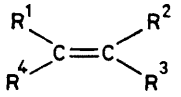


(3)

A thorough investigation of the broth of *Nodulisporium hinnuleum* led to the isolation, by preparative g.l.c., of the alcohols (**2**)—(**7**) whose structures are reminiscent of sterol side chains. The major metabolites were (**3**) and (**5**). The rotations of the alcohols (**2**) and (**3**) showed that they have

the same chirality as those derived from ergosterol.[4] The structures of the alcohols (**6**) and (**7**) were confirmed by synthesis from methyliso propyl ketone.



(**4**) $R^1 = R^2 = R^4 = H$, $R^3 = C(Me)(OH)–CH(Me)_2$
(**5**) $R^1 = R^4 = H$, $R^2 = CH(Me)_2$, $R^3 = CH_2–CH_2OH$
(**6**) $R^1 = CH_2OH$, $R^2 = CH(Me)_2$, $R^3 = Me$, $R^4 = H$
(**7**) $R^1 = CH_2OH$, $R^2 = Me$, $R^3 = CH(Me)_2$, $R^4 = H$

If these alcohols represent the side chain of a demethoxyviridin precursor, they would be expected to bear two labels from [2-^{14}C]mevalonate, whilst the demethoxyviridin should retain three labels and the ratio of the specific activities should be 2:3. [2-^{14}C]Mevalonic acid (MVA) was incubated with *Nodulisporium hinnuleum* and the results of two separate experiments are given in the Table. The ratio of the specific activities of the alcohols and of demethoxyviridin is indicative of a common ancestry.

The isolation of these fragments at the alcohol-oxidation level poses a biosynthetic question. An alcohol might arise directly by, for example, rearrangement of a C(20) hydroperoxide or indirectly *via*, for example, a C(20),C(22) diol, an aldehyde, and then reduction. Some insight into this sequence was obtained by feeding [2-$^2H_2$]mevalonic acid to *Nodulisporium hinnuleum*. Two centres in the alcohols (**4**) and (**5**) [C(1) and C(5)] should be labelled in a ratio of 2:2 or 1:2. Examination of the ^{2}H n.m.r. spectra

TABLE.

Compound	Specific activity/d.p.m. mmol^{-1}	
	Expt. 1	Expt. 2
(**1**)	119,000[a]	64,346[b]
(**3**)		38,396
(**5**)	87,000	44,232[c]
(**6**)		42,522
(**7**)		39,900

[a] 2·3% incorporation of MVA. [b] 1·17% incorporation of MVA. [c] The alcohol (**5**) was further purified as its crystalline 3,5-dinitrobenzoate which had a specific activity of 43,300 dpm mmol.$^{-1}$

of the alcohols showed that signals at δ 3·6 and 0·825 in (**3**) and δ 3·7 and 1·02 in (**5**) were in the ratio 0·6:2 and 0·7:2, respectively, suggesting that a label is lost from C(1) during the formation of the alcohols. Since [2-$^2H_2$]mevalonate was used as a substrate, the C(22) of a sterol precursor will bear either two protons or two deuterons. Hence any isotope effect in the cleavage sequence [*e.g.* in the hydroxylation at C(22) prior to the formation of the alcohol] will be reflected in a reduction in the amount of deuterium label retained in the 'inert' position at C(1) in the alcohols.

The structure of the alcohols, their specific activity per centre compared with demethoxyviridin, and the [^{2}H] labelling pattern of the two major-product alcohols (**4**) and (**5**) strongly suggest that the biosynthesis of this fungal 17-keto-steroid follows a mammalian rather than a microbial side-chain cleavage sequence.

We thank Dr. C. Turner (Varian Associates) for ^{2}H n.m.r. spectra.

(*Received*, 30*th May* 1980; *Com.* 580.)

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